UTILITY OF KI67 IN PREDICTING RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN WOMEN WITH LOCALLY ADVANCED BREAST CANCER AT THE KENYATTA NATIONAL HOSPITAL.

MASTER OF MEDICINE (M. MED) IN GENERAL SURGERY

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ABSTRACT

Background: In Kenya, majority present with locally advanced breast cancer. Neoadjuvant chemotherapy(NACT) is useful in management of these patients, and is useful in predicting response to treatment. Ki67 levels have good prediction and prognosticating value in breast cancer treatment. There is limited data on Ki67 in Kenya. This study deciphers the role of Ki67 in predicting response to NACT.

Patients and methods:
This was a prospective cohort study carried out at the Kenyatta National Hospital. Women with locally advanced breast cancer, who met the inclusion criteria, were recruited consecutively and followed up over a period of six months from December 2017 to January 2019. Informed consent was sort and data obtained included pre-treatment tumour size by ultrasound, pre-treatment Ki67 levels, demography, tumour biology was collected.On completion of 3 cycles of first line chemotherapy, response by useof ultrasound was done. Response was defined as tumor reduction by 30% from the pretreatment size. Data was analyzed by using of SPSS (V 21.0). We analyze change in tumour size, tumour biology and compared them with pretreatment Ki67 levels.

Results:The mean age was 45.9 (SD=10.4) years.The disease status on completion of 3 cycles was response at 39.4%, stable disease 50.8% and progressive disease is 9.8%. The sensitivity and specificity of Ki67 was 70.8% and 43.2% respectively ata cut off value of 32.5%. There was no association between lymphovascular invasion and KI67 levels, $\chi 2$ (1) = 2.198, p = .138, but there was statistically significant association between perineural invasion and KI67 levels, $\chi 2$ (1) = 10.509, p = .005.

Conclusions: The cut off of Ki67 in this study is high at 39.4%.Majority of patient have stable disease on completion of the third cycle. In our study the only aspect of tumour biology that has some association with higher Ki67 is perineural invasion.

CHAPTER 1: INTRODUCTION

1.1 Background

The burden of breast cancer in Kenya is high, according to the Nairobi Cancer Registry breast cancer is the commonest malignancy among women (1). It accounts for52 per 100,000 all cancer in women. The mortality currently stands at 7 percent annually(2). Globally the burden of breast cancer is on th rise gradually(3).

Breast cancer is grouped into three i.e. early, locally advanced and metastatic disease (4–6). Locally advanced disease (LABC) is common and it accounts for more than 50% the breast cancer cases at first presentation to the hospital in Turkey and Mexico respectively (7–9). Anecdotal evidence place LABC in Kenya at two-thirds of all breast cancers(10) this was similar to figures presented by Dr Githaiga-SSK presentation Kakamega 2005. Currently in Kenyatta national hospital approximately 3 to 4 patients receive neoadjuvant chemotherapy weekly (unpublished sources). This is on average 12 to 16 patients per month and 144 to192 patients per year. Treatment of LABC involves neoadjuvant chemotherapy, surgery i.e. breast conserving surgery or modified radical mastectomy for the operable patients plus radiation therapy(11,12).

LABC poses difficulties in achieving resection margins, low chance of breast conserving surgeries and wound management(13). NACT therefore enables the surgeon to achieve the acceptable resection margins, increases operability and improves wound management(14).DNA microarrays have shown that some cancers are resistant to chemotherapy and the end result is some patients receiving unnecessary chemotherapy. DNA microarrays have been used to predict response to chemotherapy but they are expensive and not universally agreed on in terms of utility(15)

Other challenges are those of monitoring and predicting treatment response(16). The techniques that help in monitoring treatment are physical examination, imaging studies ,pathologic response and biomarkers (16). Clinical and radiological methods for predicting response have been shown to be inefficient and inadequate in predicting response to therapy(17,18).

Ki67 is a nuclear antigen which is expressed in actively dividing cells. It is a marker of cellular proliferation (19). It is produced in large amounts in all cancer tissues and therefore can be used to predict response to treatment (20). There is however, paucity of data on its utility in locally advanced breast cancer. Ki67 if well applied has good accuracy and prediction role. This study therefore aims at determining the utility of Ki67 in predicting response to neoadjuvant chemotherapy in LABC. This is cheaper compared to DNA microarrays in selecting patients likely to respond to chemotherapy and avoid unnecessary therapy(21).

CHAPTER 2: LITERATURE REVIEW

2.1 Background

2.1Breast cancer burden

Breast cancer hasa global prevalence of 25% of all cancers(22). The world prevalence of breast cancer is on the rise. The projections is that 56 million premenopausal women will be at risk of invasive breast cancer by 2030(23). The African prevalence is estimated to be 35 per 100,000 and a mortality of 48,000 annually(24). In Kenya, breast cancer has a high prevalence of 51.7 per

100,000 women, with an annual mortality of 7 per 100 patients(1). Most women in Africa still present with advanced disease to the hospitals(25).

2.2Staging and grading

Breast cancer is categorized into 3 grades i.e. grade 1-3 depending on tubular structure like formation, variation in nuclear in size and the number of mitoses(26). Low grade tumors tend to have a better outcome compared to higher grade tumors(27). Currently in Kenya there is paucity of knowledge on the breast cancer tumor grades and its correlation with KI67.

Breast cancer staging is based on the TNM (Tumor size, Nodal status and Metastasis) staging system. This system however, mainly determines the extent of the disease anatomically (28). According to the TNM stratified into stage 0 to 4, stages 3 and 4 are associated with poorer prognosis(29).

Stage 0 is tumor insitu with no nodal involvements or any metastases while stage 1 is T0 and T1 i.e. tumors up to 30mm with nodal micro metastases with no distant metastases. Stage 2 is T0 to T3 with N1 nodal involvement and absent for metastases(30). LABC or stage III disease as per TNM staging system includes stage IIIA (T0N2M0,T1,2N2M0 or T3N1,2M0) or stage IIIB (T4N0M0) and Stage IIIC Any TN3M0(31). Stage 4 is any tumor size, any nodal status but has distance metastases too.

2.3 Management of locally advanced breast cancer:

Management of breast cancer depends on the stage and clinical extent(32). Early breast disease is treated mastectomy +/- sentinel node biopsy and axillary clearance or wide excision followed by whole breast irradiation therapy/intra operative radiation therapy (33). LABC is the commonest form of breast cancer and is defined as tumors more than 50mm in its widest diameter, regardless of nodal positivity but absent metastases(34).

Experience of NACT in LABC has evolved in the last half a century since its inception. NACT has numerous benefits like in vivo assessment of therapy, the use of uninjured lympho-vascular system and early elimination of micro-metastases(35). NACT has led to increased rates of breast conserving surgeries and increased operability rate of LABC and has improved wound management(36). Some tumors are however resistance to chemotherapy meaning proper

prediction of response is needed to assess therapy(37–39). The NACT regime commonly used in our set up is adriamycin and cyclophosphamide as the first line drugs (40). The regime dosage is adriamycin 60mg/m^2 and cyclophosphamide 600mg/m^2 both given intravenously every 3 weeks for 3 cycles(41). Dose dense adjuvant chemotherapy on the other hand is usually given every two weeks.

NACT down-staging of locally advanced disease results in an increase of 7-12% on breast conservation rates. However, some patients do not however, experience sufficient response due to drug resistance and hence are not candidates for breast conserving surgery(42). These are the patients that need to be identified in order to avoid unnecessary chemotherapy.

Monitoring of response after NACT

Monitoring of response is through physical exam, radiological, pathological and biomarkers.(16)

2.4Imaging

The monitoring response to treatment is based on the world health organization guidelines and the response evaluation criteria published early 1980s and 2000 respectively. RECIST and WHO guidelines usedMRI, CT scan, Ultrasonography and x-rays(43). RECIST has been validated in many studies as a useful tool in monitoring response. The measurements which are done determines whether the disease is progressive, stable or complete pathological response(44). Progressive disease is when the increase in tumor size is more than 20% in its greatest dimension from the baseline measurements while complete pathological response occurs when there is more than 30% reduction from the baseline measurements. Stable disease occurs when there is no complete pathological response nor progressive disease(45). This study combined RECIST i.e. the use ofultrasound and tumor biology.

The WHO guidelines measured disease progress by taking two measurements i.e. bi-dimensional. This has been difficulty to reproduce in many studies. The RECIST is a departure from the use two dimension to a single dimension measurement on target lesions of the largest length where only the greatest dimension is measured(41). RECIST is thus easier to use and has been shown to have a higher reproducibility and over 97% concordance in the results from several studies(47)**.**

CT scan is the gold standard tool in measuring dimensions, use of ultrasound is however, allowed due to higher radiation exposure with the use of repeated CT scans(48). The utility of CT scan depends on the type of CT scan machine available and the CT scan slice thickness since some scanners can miss lesions less than 3mm(43).

Ultrasound is available in many centres in Kenya it has no radiation exposure, it is cost effective and therefore can be an adjunct to clinical measurements in monitoring response(48). Ultrasonography has been shown to be superior to breast palpation and mammographic techniques(49). The sensitivity of ultrasound in monitoring response is comparable to that of MRI in experienced hands at 79 % vs. 84% with a P value of 0.01 (50).

A study using MRI in monitoring response in patients with Estrogen receptor positive and HER2 negative breast cancer showed that after 3 cycles of Adriamycin and cyclophosphamide if there was no response chemotherapy was changed to doxetacel and capacitamine in resistant patients. These patients had adequate response by the 3rd cycles of the new regime (51).

2.5Pathologic monitoring

Tumor biology entails the properties of the cells that aid in cellular growth and interaction of external factors that cause the development of the breast malignancies(52). Breast cancer has been stratified according to the receptor status into Luminal A, Luminal B, Basal and HER2 subtypes(53). Luminal A is estrogen receptor (ER) and progesterone receptor (PR) positive and HER 2 receptor negative. Luminal B is a triple positive tumor i.e. ER/PR/HER2 positive while Basal type is TBNC. The HER2 subtype is either positive or negative HER2 receptor status (54). The receptor status in sub Saharan Africa is comparable to that seen in other parts of the world(55).

Ingolf etal 2014 showed that patients with TNBC and HER2 subtypes which are highly proliferating have complete pathologic response. Ki67 has limitations in that it cannot distinguish between good or poor prognosis in Luminal A and B breast cancers(56).

Other modes of monitoring therapy are use of resection margins where the tumor can be R0, R1 or R2. R0 denotes complete resection, R1 microscopic residual tumor while R2 is a macroscopic residual tumor(16,57).

Biomarkers are protein found in serum or in the breast cancer cells which can be used to detect or prognosticate breast cancer(58). This have been known to influence the cancer growth rates and response to therapy(59,60).

The use of biomarker is one of the newer modes of predicting response and key among them are ER, HER2, PR and Ki67(61). Many markers that have been developed include mammastrat, mammaprint, endopredict and oncotype dx and many others (62). It is worth to noting that their clinical and analytical validity have not generally been agreed upon (62). Most of these biomarkers are not currently available in our setup due to cost and technological limitations(63). This study therefore explores the value of Ki67 in predicting treatment progress.

2.6Ki67

Ki67 is a proliferation marker expressed by actively dividing cells. It was discovered in German in 1980s. 'Ki' for Kiel University in German and 67 for well number 67 in a 96- wells plate (64). KI67 is a protein in the body marked by the gene mKi67 this antigen is usually can be targeted with monoclonal antibody (59). It is produced by many carcinomas and has been shown to correlate with the clinical course of disease (65–68).

In breast cancer, measurement of Ki67 has been recommended prior to initiating neoadjuvant therapy (69). Values more than 25% have been shown to have a favourable response to NACT(64,70,71). None of the studies has been conducted in our local set up.

Despite its potential utility in monitoring treatment response, almost all the studies on KI67 have been retrospective and the methods used to measure it had not been standardized(72). Inconsistencies in the studies resulted from the use of different treatment protocols, unspecified patient follow up time and use of different Ki-67 cut offs in determining treatment responses (72,73).

Estrogen receptor has two subtypes i.e. alpha and beta. Paradoxically, some breast tumors lack estrogen alpha subtype or Ki67 expression(74) Increased KI67 positive and estrogen alpha

receptor negative tumor are likely to have a higher tumor grade. A study by Inwald etal in 2013, showed that Ki67 can be a unique prognostistication parameter both for disease free survival and overall survival(65). A retrospective study done at Gachon University in South Korea between 2007 and 2012 concludes that a cut off of greater than 25 percent is to be used to make treatment decisions and that Ki67 alone is an independent predictor of pathological complete response(70).

The International Ki67 breast cancer working group has given recommendations to standardize and increase reproducibility of the results. Among the recommendations are to use the mouse Monoclonal antibody-1(MBA 1), use of similar tissues i.e. cores or incisional biopsy. One core biopsy is adequate in determining the Ki67 levels. They recommend the use of 10 high power fields assuming an average number of 100 cancer cells per field to get the average number staining cells and the use of immunohistochemistry to study KI67 with specimen analysis within 2 weeks(72).

Measurement of Ki67 can be done on core biopsy strips or from the excised breast or lumpectomy specimen. The Ki67 is defined as percentage of total number of tumor cells with nuclear staining this proliferating cells stain brown are counted out of 100 cells per high power field and converted to a percentage. KI67 has been shown to be important in selecting the addition of chemotherapy to endocrine therapy in hormone receptor positive breast tumors(72). The values and utility of KI67 in predicting response is not known in our setup. Worldwide where they have the values its utility is still questionable or it was done without following the laid down methodologies thus there is still need to do evaluation on Ki67.

2.7 STUDY JUSTIFICATION/RATIONALE

The world prevalence of invasive breast cancer will be 56 million by 2030 in premenopausal women(23). Therefore this could lead to a high disease burden of LABC which is complicated by lack monitoring tools that are universal(34). The potential use of KI67 in vivo in monitoring response in cancers makes it a good tool, and many studies have shown it can independently predict response to treatment (70,75). Studies done previously have been retrospective and have yielded different cut offs hence a prospective study needed for more conclusive results(72). Ki67 levels may influence early treatment adaptation and hence reducing cost and side effects of chemotherapy(51,61). It is a fact that some tumors are resistance to chemotherapy hence this will help to avoid wasting time on unnecessary therapy and its associated adverse effects(76). DNA microarrays are a better option but they are not feasible due to cost (77). Anecdotal evidence shows resistance to chemotherapy and hormonal therapy hence the need to accurately predict and monitor treatment response(78).

2.8 RESEARCH QUESTION

What levels of KI67 predict response to neoadjuvant chemotherapy in locally advanced breast cancer?

2.8OBJECTIVE

2.8.1 Main Objective

To determine the utility of KI67 in predicting response to neoadjuvant chemotherapy in women with locally advanced breast cancer in a tertiary health care facility in Kenya?

2.8.2SPECIFIC OBJECTIVES

1. Determine the correlation between ki67 levels and response to NACT based on Sensitivity and specificity, cut off and area under the curve for KI67.

2. Determine the proportion of high and low KI67 levels before neoadjuvant chemotherapy on core biopsies.
3. Determine the mean changes in tumor size pre and post neoadjuvant chemotherapy using Ultrasonography in centimetres.
4. Correlate levels of KI67 with tumor grade and receptor status
5. To determine pathologic complete response versus Ki67 levels.

CHAPTER 3: METHODOLOGY

3.1 Study setting

Kenyatta National Hospital Surgical wards, surgical outpatient clinic, oncology clinics, radiology department and histopathology laboratory.

3.2 Study population

Patients with locally advanced breast cancer who are staged clinically with CT scan abdomen, chest or chest x-ray or abdominal Ultrasonography and negative for metastases and have baseline work up urea, creatinine and electrolyte and normal complete blood count.

Patients with Locally advanced breast cancer i.e. T3 tumors (more than 50mm) regardless of nodal status but have no metastases were recruited through consecutive sampling. An informed consent wasadministered and the data was collected using a pretested and structured questionnaire. Data to be collected include Pretreatment KI67 levels, demography, histology (tumor grade, lymphovascular invasion and Immunohistochemistry i.e. ER, PR and HER2).
Tumor size was determined by ultrasound prior to therapy and at the end of 3 cycles. The research used the same Ultrasound Machine Aplio 400 with a high frequency linear probe of 12MHz for all sixty-one patients. Standard neoadjuvant first line chemotherapy which is a combination of Adriamycin 60mg/m^2 and cyclophosphamide 600mg/m^2 both given intravenously every 3 weeks(41).

3.3 Study design

A prospective cohort study

3.4 Study duration

The study was conducted between December 2017 to January 2019

3.5 Sample size

Sample size was calculated using the (Daniel, 1999) formula;

$$n=\frac{Z^2 x P(1-P)}{d^2}$$

Where,

n = Desired sample size

Z = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI)

P = expected true proportion (estimated at 0.83, from a study of the Sudanese population and published in the Ethiopian Journal of Health Sciences., (20XX), found that the clinical response rate for neoadjuvant chemotherapy at 83%.

d = desired precision (0.05)

$$n_0 = \frac{1.96^2 \, x \, 0.83(1-0.83)}{0.05^2} = 217$$

Currently in Kenyatta national hospital approximately 3 to 4 patients receive neoadjuvant chemotherapy weekly. This amounts to 12 to 16 patients per month and 144 to192 patients per year. Adjusting the sample size for finite populations less than 10,000

$$\frac{12+16}{2} = 14 \, patients \, per \, month$$

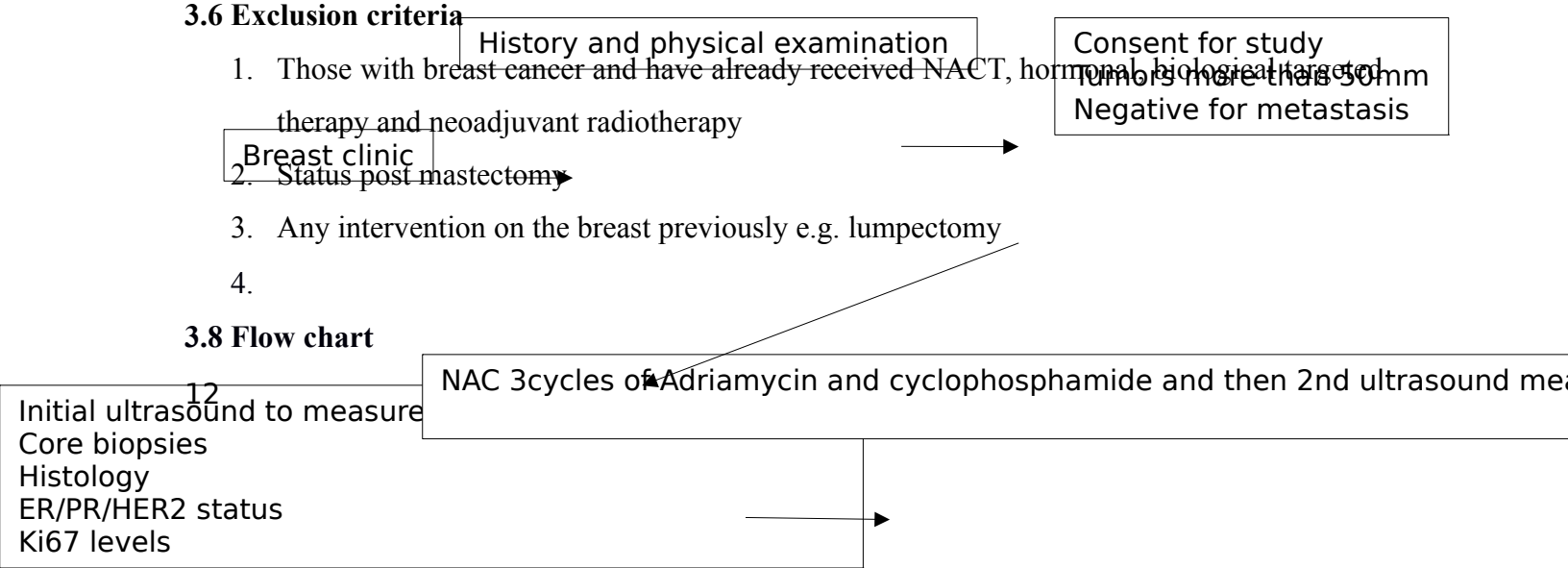
The study was done for 6 months and patients followed till they finished 3 cycles of chemotherapy, therefore a total of 84 patients are likely to be seen

$$nf = \frac{n_0}{1+\frac{n_0-1}{N}} = \frac{217}{1+\frac{217-1}{84}} = 6$$

3.6 Exclusion criteria

1. Those with breast cancer and have already received NACT, hormonal, biological,targeted therapy and neoadjuvant radiotherapy
2. Status post mastectomy
3. Any intervention on the breast previously e.g. lumpectomy
4.

3.8 Flow chart



One research assistant collected the data in pretested data sheet. He was briefed on study objectives and methodology. Patients were recruited from casualty and surgical outpatient clinics by consecutive sampling. An written informed consent was obtained from the patient or guardians. The researcher and research assistants then collect data from consenting patients.

Determination of KI67 levels from core biopsy by processing with immunohistochemistry using KI67 international breast cancer working group protocol as shown in table below(72).

Recommendations for Ki67 assessment in breast cancer

Preanalytical

Core-cut biopsies and whole sections from excision biopsies are acceptable specimens; when comparative scores are to be made, it is preferable to use the same type for both samples (egg, in presurgical studies).

- TMAs are acceptable for clinical trial evaluation or epidemiological studies of Ki67.
- Fixation in neutral buffered formalin should follow the same guidelines as published for steroid receptors.
- Once prepared, tissue sections should not be stored at room temperature for longer than 14 days. Results after longer storage must be viewed with caution.

Analytical

- Known positive and negative controls should be included in all batches; positive nuclei of nonmalignant cells and with mitotic figures provide evidence of the quality of an individual section.
- Antigen retrieval procedures are required. The best evidence supports the use of heat-induced retrieval most frequently by microwave processing.
- The MIB1 antibody is currently endorsed for Ki67.

Interpretation and scoring

- In full sections, at least three high-power (×40 objective) fields should be selected to represent the spectrum of staining seen on initial overview of the whole section.
- For the purpose of prognostic evaluation, the invasive edge of the tumor should be scored.
- If pharmacodynamics comparisons must be between core cuts and sections from the excision, assessment of the latter should be across the whole tumor.
- If there are clear hot spots, data from these should be included in the overall score.
- Only nuclear staining is considered positive. Staining intensity is not relevant.
- Scoring should involve the counting of at least 500 malignant invasive cells (and preferably at least 1000 cells) unless a protocol clearly states reasons for fewer being acceptable.
- Image analysis methods for Ki67 remain to be proven for use in clinical practice.

Data handling

- The Ki67 score or index should be expressed as the percentage of positively staining cells among the total number of invasive cells in the area scored.
- Statistical analysis should take account of the log-normal distribution generally followed by Ki67 measurement.

Determination of the change in tumor size was done using the RECIST version 1.1 protocols(44)

The breast ultrasound was done by various consultant as per the hospital policy, requiring all breast examinations by ultrasound to be done by consultant radiologists. The Ultrasound was done before induction with neoadjuvant chemotherapy and repeated at the end of 3 cycles of Adriamycin 60mg/m^2 and cyclophosphamide 600mg/m^2. Pathological complete response was done on the core biopsies.

3.9Data Management/Analysis

Data was entered cleaned and analyzed by use of SPSS (Version 21.0, Chicago-Illinois).We analyzed the; age range, mean age, and mean tumour size change from pretreatment to after 3 cycles of NACT. We analyzed the proportions of patient with high or low Ki67 according to St Galen classification, and proportion of patient who had complete pathological response. We used Chi-square to determine the association between Ki67 level to that of tumour biology aspects.

Receiver operator curve as drawn to determine the sensitivity, specificity and Ki67 cut offs and area under the curve for our population. P-values and 95% confidence intervals (CIs) were calculated as applicable. A P- value <0.05 considered statistically significant.

3.10 Ethical consideration

This study commenced upon approval from the Department of surgery and the UoN-KNH ERC P247/05/2017. Patients were enrolled after obtaining an informed consent. Patients' hospital file number were included into the data sheet to facilitate easy tracing and capture missed information during data collection.The data sheet was kept safely with the researcher and confidentiality maintained throughout the study.

Study limitations

The study was done using a finite population, a smaller sample size.

Inter observer variability on when conducting the ultrasound, it was done by various consultants.

The duration of study was also short i.e. 3 cycles.

CHAPTER 4: RESULTS

4.1 Introduction

The findings of the study are presented in this chapter.
The main objective of the study was to determine the utility of KI67 in predicting response to neoadjuvant chemotherapy in women with locally advanced breast cancer in a tertiary health care facility in Kenya.

A total of sixty-one patients were recruited and followed each for six months between December 2017 to January 2019.

4.2 Demographic characteristics

This section describes the characteristics of the women who received neoadjuvant chemotherapy at the Kenyatta National Hospital. Means and standard deviations are presented as Mean (SD) where applicable.

Table 1: Patient and tumour characteristics

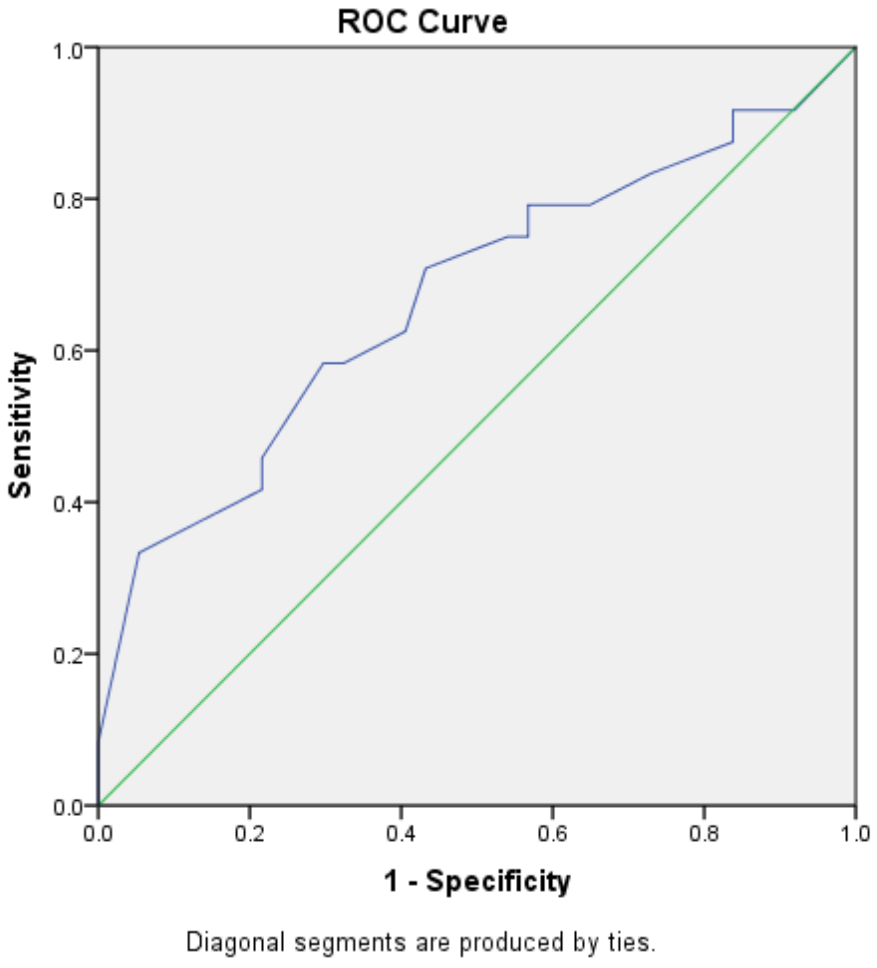
The Patient characteristics is as shown by the table below.

	Frequency n (%)
Age (years)	
≤30	4 (6.6)
31-40	15 (24.6)
41-50	27 (44.3)
51-60	8 (13.1)
61-70	6 (9.8)
71-80	1 (1.6)
Tumor Biology	
Grade	
I	10 (16.4)
II	39 (63.9)
III	12 (19.7)
Molecular type	
Luminal A	21 (34.4)
Luminal B	15 (24.6)
Basal like	19 (31.1)
HER2-enriched	6 (9.8)

The patient characteristics show that of the 61 patients, 27 (44.3%) of them belonged to the 41-50 years of age. The mean age of the patients was 45.9 (10.4) years, while the minimum age was 28 years, and maximum being 73 years. All the patient had invasive ductal carcinoma.

Figure 1: **The Receiver operator curve**.

The correlation between ki67 levels and response to NACT based on Sensitivity and specificity, cut off and area under the curve for KI67.



Area Under the Curve					
Test Result Variable(s)	**Area**	**Std. Error**	**Asymptotic Sig.**	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
KI67 Levels prior to therapy (%)	.671	.074	.025	.536	.816

	Cut off	Sensitivity	Specificity
KI67 Levels prior to therapy	32.5	0.708	0.432

Table 2: **The proportion of high and low KI67 levels before neoadjuvant chemotherapy on core biopsies.**

Ki67 levels	No. of patients	%
<20	14	23.3
>20	47	76.7

Table 3: **The mean changes in tumor size pre and post neoadjuvant chemotherapy using Ultrasonography in centimetres.**

	Mean	**SD**	**P value**
Size of breast mass (cm) by Ultrasonography at week 0	5.95	4.34	<0.001
Size of breast mass (cm) greatest dimensions at end of 3 cycles of NACT	4.71	4.07	

The paired sample mean difference was 1.24 (SD=2.19) with CI of 0.68-1.80, t (60) = 4.420, p<0.001. To test the hypothesis that the size of the breast mass (cm) by ultrasonography at week 0 (*M=5.95, SD=4.34*) and the size of the breast mass (cm) greatest dimensions of 3 cycles of NACT (*M=4.71, SD=4.07*) were equal, a dependent samples t-test was performed. It is worthy noting that the correlation between the two conditions was estimated at r=0.866, p<0.001, suggesting that the dependent samples t-test is appropriate in this case. The null hypothesis of equal breast mass means was rejected t (60) = 4.42, p<0.001. Thus, the mean of size of breast mass (cm) greatest dimensions at end of 3 cycles of NACT was statistically lower than the mean of size of breast mass (cm) by Ultrasonography at week 0.

Table 4: Ki67 Levels with Tumor Grade and Receptor Status

TheCorrelation of KI67 levels with tumor grade and receptor status

This section presents the correlation between levels of KI67 with tumor grade and receptor status.

% Ki67	≤20	>20	**OR (95% CI)**	**P value**

Well	2(14.3)	8(17)	0.9(0.2-4.8)	1.00
Moderate	9(64.3)	30(63.8)	1.0(0.3-3.5)	1.00
Poor	3(21.4)	9(19.1)	1.2(0.3-5.0)	1.00
Luminal A	3(21.4)	18(38.3)	0.4(0.1-1.8)	0.324
Luminal B	3(21.4)	12(25.5)	0.8(0.2-3.3)	1
Basal	5(35.7)	14(29.8)	1.3(0.4-4.6)	0.747
Her2-enriched	3(21.4)	3(6.4)	4(0.7-22.6)	0.128

None of the values is significant from each other.

Table5: Various responses to neoadjuvant therapy

The pathologic complete response versus Ki67 levels

	Frequency n (%)
Stable	31 (50.8)
Response	24 (39.4)
Progressive	6 (9.8)

Table 6: KI67 Levels before Neoadjuvant Chemotherapy

The KI67 levels of the women before neoadjuvant chemotherapyis as shown by the table below.

	Non-Responders	**Responders**	**OR (95% CI)**	**P value**
≤20	10 (27.0)	4 (16.7)	1.9 (0.5-6.8)	0.347
>20	27 (73.0)	20 (83.3)		

A chi-square test for proportion was conducted between the Ki67 levels and Response. There were no statistically significant differences in the proportions between Ki67 levels and Responders and non-responders, $\chi 2$ (1) = 0.884, p = .347.

Table 7: **Response rates in relation to receptor status.**

	Non-Responders	**Responders**	**Total**
ER+	24 (68.6)	11 (31.4)	35/61(57.4)
PR+	23 (79.3)	6 (20.7)	29/61(47.5)
HER2+	17 (63.0)	10 (37.0)	27/61(44.3)

Table 8: **Relationship between Ki67 and perineural and lymphovascular invasion**

	≤20	**>20**	**OR (95% CI)**	**p-value**
Lymphovascular invasion				
Present	4 (14.3)	24 (85.7)	0.4 (0.1-1.4)	0.138
Absent	10 (30.3)	23 (69.7)		
Perineural invasion				
Present	5 (71.4)	2 (28.6)	12.5 (2.1-74.8)	0.005
Absent	9 (16.7)	45 (83.3)		

A chi-square test for association was conducted between the lymphovascular invasion and perineural invasion with the KI67 levels. There was no statistically significant association between lymphovascular invasion and KI67 levels, $\chi 2$ (1) = 2.198, p = .138, but there was statistically significant association between perineural invasion and higher KI67 levels, $\chi 2$ (1) = 10.509, p = .005.

Table 9: **Molecular type vs the tumor grade**.

	Luminal A	Luminal B	Basal like	HER2-	P value

				enriched	
Well differentiated	3 (14.3)	5 (33.3)	2 (10.5)	0 (0.0)	0.133
Moderate differentiated	11 (52.4)	7 (46.7)	15 (78.9)	6 (100.0)	
Poorly differentiated	7 (33.3)	3 (20.0)	2 (10.5)	0 (0.0)	

A Fisher's exact test was performed and p-value of 0.133 (Not Significant)

CHAPTER 5:DISCUSSION

4.6.0 Demographics

Multiple studies have been conducted world over in search of predictors of response in the treatment of breast cancer patients. Ki67 is a molecule which has been studied extensively but not conclusive in its findings. It has been shown to have regional variation in its cut offs for predicting response.

The study included 61 women all with locally advanced ductal carcinoma. The mean age at diagnosis 45.9±10.4years.From multiple studies the median age at diagnosis is 48.5years in Kenya and 64.1 years among the US born Americans(79). Astudy conducted in Aga Khan University, Nairobi concluded that the median age at diagnosis was 47.5years(80).

The median age at diagnosis of breast cancer in the African population is 46years ± 6.2 S.D(81). The age commonly diagnosed with breast cancer is in the African population is 35-49 years this was found in the 20 African countries with cancer registries(82). Our study findings on age and commonest age group at diagnosis are within the various studies.

4.6.1Ki67 index, sensitivity, specificity and area under the curve

Ki67 levels has been documented in various studies to correlate with both clinical and pathologic response to NACT thus can be used in selecting patients who will benefit from the therapy. Cut off values vary widely with different patient populations and the type of NACT used(83). In our study a Ki67 cut off value of 32.5% was obtained with a sensitivity and specificity of 70.8% and 43.2% based on the ROC curve analysis. This value is close to that reported Jain et al (2019) who conducted a study among 134 patients with Stage II/III breast cancer. The study reported Ki67 as an independent predictive factor for response and suggested 35% as best cut off for Ki67 expression in predicting response to NACT and achievement of pathologic complete response. The sensitivity was 68.7 and specificity 71.6%. However, the clinical complete response rate differs from this study, there sensitivity is comparable to ours albeit our lower specificity. The response rate observed by Jain and colleagues was lower at 26.1% compared to the current study (39.3%), Jain et al study is among the few prospective studies conducted it had twice our sample size. The results could be comparable if we had a larger sample size and a longer duration of study, the also followed patients till the completion of NACT(84).

Kim Ki et al 2014 demonstrated a cut off value of 25% (Sensitivity 71%, specificity 77%) especially in ER- and HER2+ tumors with ki67 being the only independent predictor while a recent study by Chen et al (2018) reported a value of 25.5% (46.6% sensitivity, 69.9% specificity), this was a retrospective study as opposed to what the international Ki67 group recommends in terms of analysis of Ki67(70,85). The clinical complete response (8.1%) also differed from this study(70). A study by Acs B and friends reported that NACT is more efficient in tumors with at least Ki67 20% with a 95.7% sensitivity and 54.3% specificity, a cut off value lower than our study. The study also demonstrated that ki67<30% predicts better overall survival and prognosis and that if a tumor is non responsive to NACT, a high ki67 level is a poor prognostic marker(86). A similar cut off value of 20% was reported by another study (87,88). Balmativola et al reported a lower value of 18% for differentiating non responsive from responsive tumors(89). A cut off of 10% was found to be prognosticate and discriminative in a cohort with a median follow up of 183 months (90). Ki67 cut off values therefore vary in different studies. Denkert et al suggested that ki67 levels are a continuum variable, they proposed that cut off points are context dependent and that there are three groups of tumors: Tumors with low ki67 and good outcome, high ki67 had good outcomes only when the tumor responded to NACT(91).

The present study showed a higher sensitivity and lower specificity which is in accordance with the study by Acs B et al, while other studies report a higher specificity and lower sensitivity (70,84–86).

4.6.2Tumor grade and ki67 levels

The current study showed statistically significant correlation between perineural invasion and KI67 levels. This result means that the relationship of the tumor and nerves is not passive, but there are neurotropic agents released by the tumor. Perineural invasion can exist independently without lymph nodes or blood vessel invasion(92). The incidence of perineural invasion is ten times less than lymphovascular invasion, it is associated with poor prognosis(93)However, there was no statistically significant association between lymphovascular invasion and KI67 levels(94) The subtle differences in our study could be as a result of a smaller sample size hence further

studies will be needed. Contrary to these findings, Kilickap et al reported that there was no relationship between perineural invasion and ki67 positivity. However, they reported that higher ki67 levels are associated with unfavorable prognostic factors including higher grade, ER negativity, Her2 positivity and axillary lymph node involvement(95).

Our study showed no statistically significant association between ki67 levels and tumor grade. Awadelkarim KD and co-workers in Sudan suggested positive correlation between ki67 and tumor differentiation but it wasn't linked to any other variables tested including receptor type and tumor size(96). According to Ragab et al, Ki67 scores increases with increase of tumor size, tumor grade, lymph node positivity, PR negativity and ER negativity(97). Similarly, Haroon S et al conducted a study among 194 cases of newly diagnosed breast cancer with an aim of correlating ki67 expression with other prognostic markers including tumor grade. The workers reported that ki67 is positively correlated with histological grade and negatively correlated with ER and PR content. The study also elucidated that high ki67>30% shows a lower HER2neu expression and lymph node metastasis hence may have better prognosis(75).

4.6.3Tumor Size Pre-and-Post Neoadjuvant Chemotherapy

The present study showed that the mean of size of breast mass greatest dimensions at end of 3 cycles of NACT was statistically lower than the mean of size by Ultrasonography at week 0. NACT, introduced in the 1980s is the standard of care for LABC as it downgrades the size of the tumor, enabling surgery to be performed(98). Complete pathologic response acts as a marker of survival following NACT. Response rate varies from 15-30% (99). Our study reported a higher response rate of 39.3%.

A meta-analysis with a patient population of 4756 women confirmed that NACT results in higher rates of breast conserving therapy. However, they reported a small increase in local recurrence with NACT but not distant recurrence and effect on overall survival(100).

The result demonstrates that Ki67 can be used as a predictor of response especially with high levels of ki67. That we should continue doing ki67 on all histological specimens because it is relevant to treatment outcomes.

Response to treatment cannot be pegged on the correlation of Ki67 and the tumor receptor status or the molecular subtypes since ki67 is part of the molecular subgroups. The mean change in the

breast change post therapy demonstrates that there is overall good benefit; thence we should continue administration of neoadjuvant therapy.

Interobserver variability during ultrasonography. The study sample size was adjusted for the finite population meaning they are relevant at the KNH was the main study limitations.

There was no Herceptin for more than 5m months during the duration of our study hence a good number of patients mainly Her2 subtype were taken directly for surgical intervention without NACT.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

Conclusion

The cut off of Ki67 in this study is higher than other studies. Majority of patient have stable disease by the third cycle. In our study the only aspect of tumour biology that has some association with higher Ki67 is perineural invasion.

Recommendations

Studies with a larger sample size required.Longer duration of study i.e. up to six cycles needs to be conducted to further delineate our cut off values.

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